<Division of Environmental Chemistry> Molecular Microbial Science

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- Molecular Microbial Science -

http://www.kuicr.kyoto-u.ac.jp/labos/bm2/lab_J.html

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Scope of Research

Structure and function of biocatalysts, in particular, pyridoxal enzymes and enzymes acting on xenobiotic compounds, are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of sulfur, selenium, and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, molecular structures and functions of psychrophilic enzymes and their application are under investigation.

Research Activities (Year 2005)

Presentations

An Enzyme Usuful for the Synthesis of Optically Active *N*-Alkyl Amino Acids and Cyclic Imino Acids, Mihara H, Muramatsu H, Kakutani R, Yasuda M, Ueda M, Kurihara T, Esaki N, The 57th Annual Meeting, Vitamin Soc. Jpn., 27 May.

Iron-sulfur Cluster Assembly by Suf Proteins of *Escherichia coli*, Kazuoka T, Mihara H, Kurihara T, Esaki N, 2005 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 30 March.

Morphological Changes of a Psychrotrophic Bacterium, *Shewanella* sp. Ac10, Depending on Cultivation Temperatures, Kawamoto J, Kurihara T, Kitagawa M, Asada K, Esaki N, 2005 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 30 March.

Mechanism of Selenocysteine Lyase from Rat, Kurokawa S, Mihara H, Kurihara T, Esaki N, 2005 Annual Meeting, Jpn. Biochem. Soc., 20 October.

Detection of Phosphatidylthreonine in Porcine Brain and It's Localization, Omori T, Kazuoka T, Mihara H, Kurihara T, Esaki N, 2005 Annual Meeting, Jpn. Biochem. Soc., 21 October.

Identification of Amino Acid Residues Essential for Hydrolytic Defluorination by Fluoroacetate Dehalogenase, Jitsumori K, Kurihara T, Omi R, Miyahara I, Hirotsu K, Esaki N, 2005 Annual Meeting, Jpn. Biochem. Soc., 20 October.

Grants

Esaki N, Dynamics of an Essential Trace Element, Selenium, in Mammals and the Molecular Basis of Selenoprotein Biosynthesis, Grant-in-Aid for Scientific Research (B),

Structure and Reaction Mechanism of Fluoroacetate Dehalogenase

Fluoroacetate dehalogenase from *Burkholderia* sp. FA1 (FAc-DEX FA1) catalyzes the hydrolytic dehalogenation of haloacetates. Although the carbon-fluorine bond is stronger than other carbon-halogen bonds, the enzyme shows the highest activity toward fluoroacetate. The activities toward chloroacetate and bromoacetate were less than 5% of the activity toward fluoroacetate. In order to elucidate the reaction mechanism of the enzyme, we determined the three-dimensional structures of the native FAc-DEX FA1, the D104A FAc-DEX FA1 complexed with the substrate, and the H271A FAc-DEX FA1 ester-intermediate. We found that Arg105, Arg108, His149, Trp150, and Tyr212 had short contacts with the substrate fluoroacetate. When Trp150, which interacts with the carboxylate group of the substrate, was replaced with Ala, the activity toward fluoroacetate was completely lost: the K_m and V_{max} values of the wild-type enzyme for fluoroacetate were 9.1 mM and 61 μmol/min/mg, respectively, whereas the W150A mutant enzyme showed no activity toward fluoroacetate. In contrast, the activity toward chloroacetate was not significantly affected by the same mutation: the K_m and V_{max} values of the wild-type enzyme were 15 mM and 2.6 μmol/min/mg, respectively, and the K_m and V_{max} values of W150A were 24 mM and 2.0 μmol/min/mg, respectively. Thus Trp150 is specifically required for the defluorination. Structural comparison between the W150A mutant enzyme and the wild-type enzyme would give us a clue for clarifying how the enzyme catalyzes the cleavage of carbon-fluorine bond.

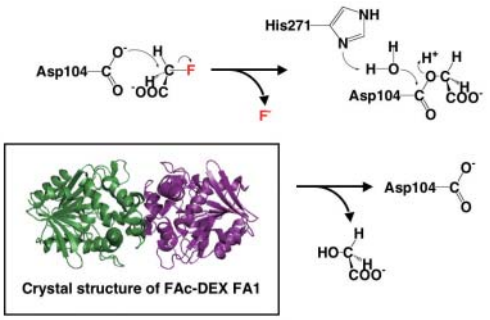

Figure 1. Structure and reaction mechanism of FAc-DEX FA1.

Enzymatic Synthesis of L-pipecolic Acid by Δ^1-piperideine-2-carboxylate Reductase from *Pseudomonas putida*

Optically active L-pipecolic acid occurs as constituents in several biologically active natural products. Therefore optically pure L-pipecolic acid is thought to be useful building blocks of medicines and pesticides. We found Δ^1-piperideine-2-carboxylate (Pip2C) reductase in *Pseudomonas putida*. Pip2C reductase catalyzes the NADPH-dependent synthesis of L-pipecolic acid from Δ^1-piperideine-2-carboxylic acid. Recombinant *E. coli* BL21(DE3) expressing both Pip2C reductase and glucose dehydrogenase was constructed. A crude extract of the cells was obtained by sonication and centrifugation. L-Pipecolic acid was synthesized in a reaction mixture containing 1.5 mg-protein/ml crude extract, 1.5 U/ml L-lysine oxidase, 14 U/ml catalase, 1% L-lysine, 100 mM glucose, 0.2 mM $NADP^+$, 1.0 mM FAD and 100 mM Tris-HCl (pH 7.5) at 30°C and with reciprocal shaking. After 5 hr, 0.5% L-lysine, 50 mM glucose and 7 U/ml catalase were added to the reaction mixture. L-Lysine and L-pipecolic acid were determined by HPLC. After 15-hr reaction, 14 g/l L-pipecolic acid was obtained (98% yield). Optical purity of the product was analyzed by HPLC and determined to be >99% e.e.

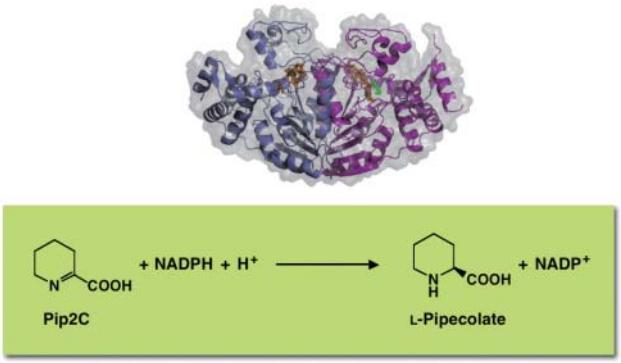

Figure 2. Crystal structure and catalytic reaction of Pip2C reductase.

1 April 2005 - 31 March 2007.

Kurihara T, Conversion of Organofluorine Compounds with Microbial Enzymes: Mechanistic Analysis of the Enzyme Reactions and Their Application to Production of Useful Compounds and Bioremediation of Environments, Grant-in-Aid for Scientific Research (B), 1 April 2005 - 31 March 2007.

Kurihara T, Exploration of Novel Cold-adapted Microorganisms That Inhabit the Polar Regions and Investigation of Their Useful Enzymes, Grant-in-Aid for Scientific Research (B), 1 April 2005 - 31 March 2007.

Mihara H, Mechanisms of Incorporation of Sulfur and Selenium into the Anticodon Wobble Bases of tRNAs, Grant-in-Aid for Young Scientists (B), 1 April 2003 - 31 March 2006.